REMARKS

Claims 36, 39, 40, 43 and 44 were previously pending in this application. Claims 36, 39, 40, 43 and 44 are pending for examination with claims 36, 40, and 43 being independent claims. Applicant notes that the word "therapeutically" was inadvertently omitted from claim 36 in the response filed April 21, 2003. Claim 36 was not amended to remove the word therapeutically therefore in the claims presented herein, the word therapeutically appears in the claim as originally filed. No new matter has been added.

Rejections Under 35 U.S.C. §112

The Examiner has rejected claims 36, 39, 40, 43, and 44 under 35 U.S.C. §112, first paragraph as containing subject matter not adequately described in the specification so as to enable one skilled in the art to practice the invention. Applicant respectfully traverses the rejection.

The Examiner states as a basis for the rejection of claims 36, 39, 40, 43, and 44 under 35 U.S.C. §112, first paragraph, that "there is no guidance at all on the selection of suitable test compounds" (Office Action at page 3). Applicant respectfully disagrees with this conclusion. The goal of a screening assay such as that taught in the instant application is for the identification of suitable compounds from a wide range of sources. It is not an assay designed solely for the identification of compounds that are related to known inhibitors, but rather it allows one of ordinary skill to assess the potential usefulness of a broad range of compounds without the restraint of beginning with "known" inhibitors. Thus, the testing of compounds from a wide range of sources, e.g., commercially available libraries of compounds etc., is an art-recognized strategy for using an assay of the invention to identify compounds that are useful in the claimed treatment methods of the invention. Applicant respectfully asserts that no additional guidance as to compound selection is required because compound screening for the identification of therapeutic compounds is standard practice for those of ordinary skill in the art of drug discovery and medicinal chemistry. Thus, Applicant asserts that the assay provided in the specification, coupled with the level of knowledge in the art, provides sufficient guidance necessary for one of ordinary skill in the art to select compounds to test using the assays of the invention.

The link between MLK activity and cell death is clearly made in the specification as filed and a considerable amount of evidence is presented in the instant application indicating that the inhibition of MLK activity results in the inhibition of cell death-associated neurodegeneration. The existence of enzyme activity inhibitors and their use in therapeutics is well known and accepted in the art. It is not necessary for the Applicant to establish the exact action of the MLK inhibition in Parkinson's disease, but rather to indicate that it would be reasonable to one of ordinary skill in the art that the MLK inhibitor compounds can be used in the treatment of Parkinson's disease. Given that the invention is clearly within the art-accepted parameters of enzyme inhibitor-based therapeutics, it is sufficient to demonstrate a reasonable expectation of success in the use of the invention by one of ordinary skill in the art.

The Examiner states that "effective treatments for disease conditions are relatively rare." Applicant respectfully submits that such an effect need not be "common"; and that the appropriate test for an enablement analysis is whether one of ordinary skill in the art would be required to use undue experimentation to practice the invention. Accordingly, requiring Applicant to have demonstrated a therapeutic effect as of filing of the application is an inappropriate standard.

Examples of the utilization of enzyme inhibition to treat disease *in vivo* are widely available in the art because many pharmaceuticals are enzyme inhibitors. Several examples of enzyme inhibitor pharmaceuticals can be found in Bjelaković et al, Competitive Inhibitors of Enzymes and Their Therapeutic Application, *Medicine and Biology*, Vol. 9, No 3, pp. 201-206, 2002 (copy submitted herewith). In addition, an example of the utilization of an enzyme inhibitor to treat a neurological condition *in vivo* is provided in Dyker, et al, Perindopril Reduces Blood Pressure but Not Cerebral Blood Flow in Patients with Recent Cerebral Ischemic Stroke, *Stroke* , 28:580-583, 1997 (copy provided herewith). This publication clearly indicates that at a time prior to the filing date of the instant application, the teaching in the art included the use of enzyme inhibitors for the treatment of degenerative neurological disorders.

The examples cited above, along with numerous other publications in the literature, clearly indicate that in the pharmaceutical arts at the time of filing the instant application, the use of enzyme activity inhibitors in therapeutic applications for disease, including degenerative neurological disease, was a matter of routine experimentation for one of ordinary skill in the art.

The instant application provides a novel target/pathway for ameliorating cell death in neurodegenerative diseases such as Parkinson's disease.

The Examiner's conclusions that "effective treatments for disease conditions are rare and may be unbelievable in the absence of strong supporting evidence," do not preclude the use of enzyme inhibitors in the treatment of neurodegenerative disease such as Parkinson's disease. The Examiner's statement purporting a scarcity of treatments for disease, appears to conflict with the existence of numerous examples of the successful therapeutic utilization of enzyme inhibitors by those in the art.

Determination of undue experimentation follows from the analysis of the eight *Wands* factors. *In re Wands* 858 F.2d 731, 737, 740, 8 U.S.P.Q.2d 1400, 1404, 1407 (Fed. Cir. 1988). It appears that only some of these factors were considered by the Examiner. Applicant maintains that full consideration of each and all of the *Wands* factors, in view of the state of the art at the time of filing, leads one to the reasonable conclusion that practicing the invention would not require undue experimentation.

The Examiner has apparently considered the predictability of the art, although perhaps using an excessively stringent standard. In contrast to the Examiner's assertions of unpredictability of biological response to therapeutic treatments, the predictability of the art as a whole for this aspect of the invention is high. One of ordinary skill in the art could reliably predict that an MLK inhibitor identified and tested using the methods provided in the specification, can be used to ascertain its ability to inhibit MLK activity and cell death. Numerous model systems (both cellular and whole organism) were available at the time of filing to test various aspects of therapeutic efficacy of putative treatments for neurodegenerative disease. For example, one could have tested the MLK inhibitor compounds in cells or animal models of Parkinson's disease to determine the effect on cell death and the Parkinson's disease phenotype. Animal models of Parkinson's disease were available at the time of filing with which one of ordinary skill could test the compounds identified with the compound assays presented in the specification. It is predictable from these and other possible routine experiments that one could determine MLK inhibitor effect and/or efficacy.

The Examiner stated that the instant specification is "absent actual working examples of how the invention would treat an individual with Parkinson's." Although it is true that no

working examples of treatment were provided in the specification, working examples of MLK inhibition as a means of inhibiting neuronal cell death (i.e., the mechanism of neurodegenerative diseases) were provided in the specification.

The Examiner did not appear to consider the remaining *Wands* factors: 1) quantity of experimentation, 2) breadth of the claims, 3) the nature of the invention, 4) the state of the prior art, and 5) the level of one of ordinary skill in the art. Applicant submits that these factors would support a finding of enablement for the claimed invention. For example, very little experimentation is required to identify, test and use MLK inhibitors for therapy of neurodegenerative disease once the method of screening for such compounds is provided, as was done in the instant application.

Applicant maintains that adequate examples and guidance were provided. Applicant provided a description of the huntingtin molecule and its use as an MLK inhibitor (see, e.g., Examples section). *In vivo* methods for testing the function of an MLK inhibitor identified using the methods described in the specification were well known at the time of filing and Applicant provided methods to test the effectiveness of identified MLK activity inhibitors *in vitro* and in cell systems as described in the Examples section. In addition, various animal models for Parkinson's disease were available and well known to those of skill in the art at the time of filing (see Parkinson's Disease Animal Models Resources, National Institute of Neurological Disorders and Stroke, at http://www.ninds.nih.gov/parkinsonsweb/amr/index.htm). The descriptions provided in the specification as filed, in conjunction with the state of the art at the time of filing, provide sufficient guidance to one of ordinary skill in the art at the time of filing (in 1998) to make and use MLK inhibitors identified using the screening assays of the invention. Thus, Applicant asserts that it would be considered routine for one of skill in the art to use such an animal model to test the efficacy of MLK inhibitors given the teaching in the specification as filed and the knowledge of one skilled in the art.

With respect to the working examples *Wands* factor, the court in *Wright* stated that "Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples." *In re Wright* 999 F.2d 1557, 1561, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993) *citing In re Marzocchi* 439 F.2d 220, 223, 169 USPQ 367, 369 (C.C.P.A. 1971). Applicant has provided not only broad terminology

which is readily understandable to one of ordinary skill in the art, but also illustrative examples as noted above. Thus the examples and guidance presented are not, by themselves, sufficient reasons to find undue experimentation.

The quantity of experimentation that would be required to practice the claimed invention is not excessive. Rather, the nature and quantity of such experimentation is completely routine in the relevant art. Use of the provided assay to identify MLK inhibitors, and testing such inhibitors, are standard experimental procedures in molecular biology. For example, given the state of the art, one of ordinary skill in the art would only use routine experimentation to identify a series of MLK inhibitor compounds and test them in cells and/or animal systems. Such experimentation is routine as shown by the cited references and numerous other references publicly available at the time of filing of the application. Accordingly, any experimentation required would not be undue.

Applicant also submits that the claims are not excessively broad. Applicant has claimed administration of MLK inhibitors identified with the disclosed assays, which reduce cell death. The nature of the invention, the use of enzyme inhibitors to treat disease, including neurodegenerative disease, is well known to one of ordinary skill in the art.

The last two *Wands* factors are important to any determination of undue experimentation. In the *Wands* case, for example, the court's decision turned on the "high level of skill in the art at the time the application was filed", and that "all of the methods needed to practice the invention were known." *Wands* at 740, 8 USPQ2d at 1406. Applicant maintains that the same conclusions with respect to the state of the art and the level of skill in the art are true in the instant case, and therefore must weigh heavily in favor of a finding that undue experimentation is not required.

The level of skill in the art has an important effect on the amount of guidance which must be provided to enable the invention. As the court stated in *In re Howarth*, "[i]n exchange for the patent, [the applicant] must enable others to practice his invention. An inventor need not, however, explain every detail since he is speaking to those skilled in the art." (emphasis added) *In re Howarth*, 654 F.2d 103, 105 (C.C.P.A. 1981). Thus the level of knowledge of one of ordinary skill in the art cannot be ignored in the *Wands* factor analysis. For the standard procedures contemplated in the application, the level of skill in the art is high. Applicant

maintains that the person of skill in the art of molecular biology or medicine would know how to identify, test, and use MLK inhibitors of the invention.

In summary, a full analysis of the *Wands* factors favors a conclusion that only routine experimentation would be required of one of ordinary skill in the art to practice the claimed invention throughout its scope. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejections of claims 36, 39, 40, 43, and 44 under 35 U.S.C. §112, first paragraph.

Rejections Under 35 U.S.C. §103

The Examiner rejected claims 36, 39, 40, 43 and 44 under 35 U.S.C. §103(a) as being unpatentable over Miller et al. (US 6,060,247). Applicant traverses the rejection.

To support a *prima facie* case of obviousness, the Examiner must demonstrate that the cited reference teaches all of the claimed features, there would be motivation to modify the teaching in the reference to make the claimed invention, and there would be a likelihood of success in making the modification. Applicant respectfully asserts that although the Examiner seems to suggest that the claimed invention was obvious to try based on the '247 patent, the Examiner has not met the standard necessary to support a *prima facie* case for obviousness of the claimed invention based on the '247 patent.

Applicant submits that although the '247 patent provides a screening assay for finding test compounds that have neuronal death and/or growth-modulating activity, the patent does not specifically teach the use of a compound that inhibits MLK activity as a treatment for Parkinson's disease. The '247 patent provides a lengthy list of diseases associated with cell death (e.g. cancer) and refers to Parkinson's disease in the Background of the Invention. In addition, the '247 patent lists various adenovirus constructs and contemplates their use in screening assays. One construct deemed relevant by the Examiner is the adenovirus construct MLK (mixed lineage kinases—SPRK, DLK, ZPK, MUK), which is described at col. 29, line 53-54. Applicant respectfully asserts that the teaching in the '247 patent fails to provide a reasonable expectation of success.

The '247 patent teaches use of adenovirus constructs to identify compounds that either increase or decrease apoptosis, but the '247 patent offers no teaching as to how one would select one versus another type of adenovirus construct to identify a compound to treat any specific

disease or disorder. The Examiner has not indicated why a skilled artisan, without knowledge of the relationship between MLK, apoptosis, and/or Parkinson's disease, would have selected one particular adenovirus construct assay from among the many disclosed in the '247 patent. Furthermore, the '247 patent does not indicate whether MLK expression is associated with the inhibition of apoptosis or with the induction of apoptosis. Therefore, the '247 does not teach or even suggest the identification and/or administration of a compound that <u>inhibits</u> MLK activity for the treatment of Parkinson's disease. Thus, the '247 patent does not provide all the elements of the invention claimed in the instant application, and the proper showing to support an obviousness rejection has not been provided.

Applicant respectfully asserts that the Examiner has failed to provide any evidence of motivation for one of ordinary skill to use the disclosures of the '247 patent to make the instant claimed invention. In particular, Applicant asserts that the '247 patent does not teach the use of compounds that inhibit MLK activity for the treatment of Parkinson's disease. First, the '247 patent does not teach that the use of any compounds identified using any specific adenovirus construct would be useful for the treatment of any particular disease. Whether or not the '247 patent provides a <u>general</u> motivation to one of ordinary skill in the art to make and use various adenovirus-based assays to identify cell death-associated compounds, the '247 patent does not provide the motivation required for a finding of obviousness. The Examiner must demonstrate a specific motivation to identify compounds that are MLK inhibitors and then apply those compounds to the treatment of Parkinson's disease, as claimed by Applicant, e.g., the '247 patent must have taught the use of the adenovirus construct to identify MLK activity inhibiting compounds and to use these compounds for the treatment of Parkinson's disease. *In re Werner Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000) ("[A] rejection cannot be predicated on the mere identification ... of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.") Applicant respectfully asserts that the '247 patent does not meet this requirement.

Applicant respectfully submits that the Examiner has not supplied any motivation or reason why one of ordinary skill in the art would select the MLK activity inhibitors for use in the treatment of Parkinson's disease from among the alternative screening assays, inhibitors and

enhancers, and diseases provided in the '247 patent. In essence, the Examiner appears to be suggesting that the claimed invention was obvious to try based on the prior art. "Obvious to try," however, is not a proper and legitimate standard for unpatentability, and does not constitute obviousness. *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1599 (Fed. Cir. 1988).

In summary, the '247 patent does not provide the elements of Applicant's claimed invention, nor does it provide the specific and clear motivation required under the law to identify MLK inhibitors and administer them to treat Parkinson's disease as required to obtain the claimed invention. The Examiner has not indicated any other source for motivation to modify the teachings of the '247 patent to obtain the claimed invention. Accordingly, Applicant respectfully requests that the Examiner withdraw the rejection of the claims under 35 U.S.C. §103 based on US 6,060,247 (Miller et al.).

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's representative at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,
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By: ______________________

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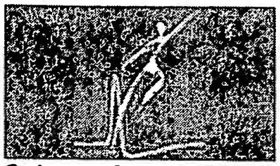
Docket No. L0624.70001US00
Date: March 30, 2004
x03/30/04x



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Priorities & Actions
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Animal model
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Related Disorders

Search NINDS... (help)
Enter Words Here Go
Contact us
My privacy

NINDS is part of the National Institutes of Health

Parkinson's Disease Research Web

Get Web page suited for printing
Email this to a friend or colleague
Join our electronic mailing list

Mouse

Chemical Lesion Models

Chemical	Administration	Phenotype	Reference
MPTP**	Systemic		
MPTP + maneb	Systemic	Exacerbation of MPTP phenotype on locomotion and catalepsy.	Takahashi, et al. Res Commun Chem Pathol Pharmacol 1989; 66(1):167-70.
MPTP + Probenicid	Systemic	Chronic loss of dopamine, gradual loss of nigral neurons. Motor decline as assessed by rotorod.	Petroske, et al. Neuroscience 2001;106(3):589-601.
6-OHDA***	Stereotactic injection	Acute nigrostriatal denervation. Dose-dependent amphetamine and apomorphine-induced circling.NOTE: effects of 6-OHDA in mice are transient.	Przedborski, et al. Neuroscience 1995; 67(3):631-47.
Paraquat	Systemic	Loss of nigrostriatal neurons and terminals. Reduced ambulatory activity.	Brooks, et al. Brain Res 1999; 823(1-2):1-10
Paraquat + maneb	Systemic	Cell loss in substantia nigra, reduced TH and dopamine.	Thiruchelvam, et al. J Neurosci 2000; 20 (24):9207-14.
3 nitrotyrosine	Intrastriatal injection	Acute striatal degeneration, TH loss, ipsilateral turning behavior with amphetamine.	Mihm, et al. J Neurosci 2001; 21 (11):RC149.

*note: combined use of pesticides or toxins tends to give enhanced phenotype.

**note: MPTP toxicity varies with different mouse strains. For review of 7 strains, see: Hamre, et al. Brain Res 1999; 828:91-103.
***note: rats more commonly used with 6-OHDA due to well-established stereotactic technique and lower costs.

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COMPETITIVE INHIBITORS OF ENZYMES AND THEIR THERAPEUTIC APPLICATION

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Summary. *Enzymes catalyze virtually every biochemical process in the cell. The usefulness of the most important pharmaceutical agents, antimetabolites, is based on the concept of competitive enzyme inhibition. The antimetabolites are structural analogues of normal biochemical compounds. As competitive inhibitors they compete with the naturally substrate for the active site of enzyme and block the formation of undesirable metabolic products in the body. Antibacterial, antiviral and anticancer pharmaceutical agents are among numerous examples of antimetabolites. Sulfa drags, sulfanilamide, structural analogs of amino acids (cycloserine, L-fluoroalanine), folic acid antagonists (4-amino-10-methyl folic acid=methotrexate), analogues of purine and pyrimidine (6-mercaptopurine, allopurinol, 5-fluorouracil, 5-azacytidine), inhibitors of polyamine biosynthesis (difluoromethyl ornithine, methylglyoxal-bis (guanyl hydrazone)) are the most used in modern chemotherapy The use of enzyme inhibitors, antimetabolites, beside the therapeutic significance has also provided valuable informations about enzyme mechanisms and has helped to define some metabolic pathways.*

Key words: *Enzyme inhibitors, sulfa drugs, antimetabolites, folic acid antagonists, purine and pyrimidine analogues, polyamines antagonists, chemotherapy*

Introduction

Enzymes are the reaction catalysts of biological systems which accelerate and direct specific biochemical reactions. Great specificity (speciality) of enzymes is a very important biological phenomen which assures high coordination to yield a harmonious interplay among many different metabolic activities necessary to sustain life.

Modification of enzyme activity

It is well known that activities of intracellulary and extracellulary enzymes depend on numerous constituents of medium or circumstances. The most important factors which influence enzyme activity are presented by enzyme concentration, the amount of specific enzyme substrate, electrochemical reaction of medium for enzyme activity (pH), the presence of activators (specific or nonspecific) as well as the presence of inhibitors (naturaly occuring or intended for specific purpose, comonly used as chemotherapeutic agents).

Inhibitors of enzymes

The inhibitors of enzyme activity are chemical substances, which in small quantity decrease the activity of enzymes in a specific chemical way. As a result of the inhibitor - enzyme interaction enzyme-inhibitor a complex is formed: once bound, the enzyme cannot convert the inhibitor to products. The existence of specific naturally occurring enzyme inhibitors, like antithrombin, antipepsin and antitrypsin, controls the enzyme activity in human the body and under physiological circumstances assures their intracellular and extracellular action. Among the naturally occurring enzyme inhibitors there are also intermediary products formed during some metabolic pathways. Product inhibition provides a limited mean of control or modulation of substrate flux through the pathway. If one or more enzymes are allosteric enzymes particularly sensitive to product inhibition, the output of end product of the pathway will be suppressed (1).

Mechanism of competitive inhibitors action

Competitive inhibitors are compounds that resemble structurally the substrate and compete with substrate for the active site of an enzyme to form an enzyme-inhibitor complex. Once the inhibitor occupies the active site of enzyme it prevents binding of substrate and abolishes the formation of normal metabolic product (1,2). Inhibitor binds reversibly the enzyme and because of that the competition can be decreased simply by adding more substrate. When enough substrate is present the

probability that an inhibitor molecule will be bound is minimized, and enzyme reaction exhibits a normal V_{max}. In the presence of competitive inhibitor Michaelis-Menten constant, K_m will increase (2).

Application of competitive inhibitors in medicine

Enzymes catalyze virtually every process in the cell and it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known. Classic example of competitive inhibitor of *succinate dehydrogenase* is malonic acid / $HOOC-CH_2-COOH$ /, structural analog of succinic acid/ $HOOC-CH_2-CH_2-COOH$ / (Fig. 1). In the presence of malonic acid succinate dehydrogenase activity, one of citric acid cycle enzymes, is inhibited, and the reaction of citric cycle is blocked, respectively (1-2).

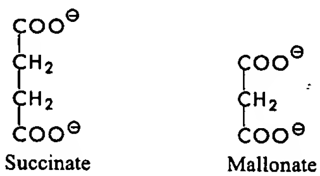


Fig. 1.

Most modern drug therapy is based on the concept of enzyme inhibition

Competitive inhibition is used therapeutically to treat patients who have ingested methanol. In the human body ingested methanol is converted into formaldehyde by the action of the enzyme alcohol dehydrogenase. Formaldehyde damages many tissues, and blindness is a common result because the eyes are particularly sensitive. The therapy of methanol poisoning is intravenous infusion of ethanol; ethanol competes effectively with methanol as a substrate for alcohol dehydrogenase. Ethanol is also substrate for alcohol dehydrogenase forming acetaldehyde and acetate. Intravenous infusion of ethanol slows down the formation of formaldehyde so that most of methanol can be excreted harmlessly by urine.

The application of therapeutical drugs as a specific enzyme inhibitors, inhibits the playing of unwanted metabolic pathways in the body and for that reason these drugs are named **antimetabolites** (2). Antibacterial, antiviral and antitumor drugs belong in the group of this drugs. The administration of those drugs to the patients causes limited toxicity because there are few critical metabolic pathways that are unique to tumors, viruses, or bacteria; hence drugs that kill these organisms will often kill host cell. Antimetabolites are compounds with some structural difference from the natural substrate and belong in the group of competitive enzyme inhibitors. Sulfa drugs, structural analogs of amino acids, folic acid antagonist, analogs of purines and pyrimidines belong to this group of enzyme inhibitors.

Application of sulfa drugs in medicine

Modern chemotherapy had its beginning in compounds with the general formula $R-SO_2-NHR$, The simplest member of sulfa drugs is sulfanilamide, an antibacterial agent. Sulfanilamide is an antibiotic useful in the treatment of some kidney infection. As a structural analog of p-aminobenzoic acid (PABA) (Fig. 2), sulfanilamide inhibits bacterial growth. PABA is a structural part of folic acid, which is composed of pteridine, p-aminobenzoic acid and glutamic acid. Some kinds of bacteria require folic acid for their growth and division. As the structural analog of p-aminobenzoic acid sulfanilamide is a competitive inhibitor for bacterial dihydrofolate synthetase. Thus bacteria are starved of the required folate and cannot grow and divide. Sulfanilamide is antibiotic useful in the treatment of some kidney infection. This drug is highly toxic to bacteria that must synthesize their own folic acid. Since humans require folate from dietary source, the sulfanilamide is not harmful at the doses that kill bacteria (2).

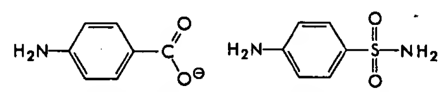
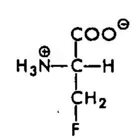


Fig. 2.

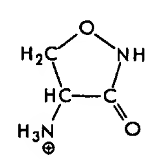
Structural analogs of amino acids

Structural analogs of amino acids are used as antibacterial drugs. D-amino acids, like D-alanine and D-glutamic acid, occur as structural part of bacterial cell walls and peptide antibiotics. D-Amino acids arise directly from the L isomers by the action of amino acid racemases, which have pyridoxal phosphate as a required cofactor. Racemisation of amino acids is uniquely important to bacterial metabolism, and enzyme such as alanine racemase represent prime targets for pharmaceutical agents. One such agent, L-fluoroalanine (Fig. 3), is being tested as an antibacterial drug. Cycloserine, analog of serine (Fig. 4), is already used to treat urinary tract infection and tuberculosis. In modern psychiatry cycloserine is frequently used as a therapeutic agent (3-5). As a structural analog of serine, cycloserine inhibits the synthesis of sphingosine, sphingomyelin respectively (6).



L-Fluoroalanine

Fig. 3.



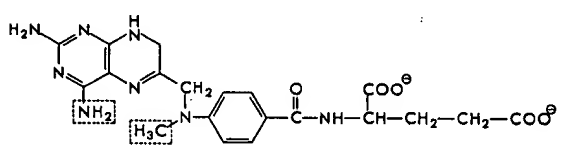
Cycloserine

Fig. 4.

Folic acid antagonists-antifolates

Folic acid, folacin or pteroil glutamic acid belong to the group of water soluble vitamins. Fresh leafy green

vegetables, cauliflower, kidney and liver are rich sources of folic acid. The physiological function of folic acid coenzymes is in the synthesis of purine nucleotides and thymine, precursors in the synthesis of RNA and DNA intracellulary, respectively. The folic acid coenzymes are specifically concerned with biochemical reactions involving the transfer and utilization of the single carbon (C_1) moiety. Before functioning as a C_1 carrier, folic acid must be reduced, first to 7,8-dihydrofolic acid (H_2 - folate) and then to the tetrahydro compound (H_4 - 5,6,7,8-tetrahydrofolic acid) catalyzed by folic acid reductase which uses NADPH as hydrogen donor.The participation of folic acid coenzymes in reaction leading to synthesis of purines and to thymine, the methylated pyrimidine of DNA, emphasizes the fundamental role of folic acid in the growth and replication of cells. Cancer cells grow more rapidly than the cells of most normal tissues and thus they have greater requirements for nucleotides as precursors of DNA and RNA synthesis. Consequently, cancer cells are generally more sensitive to inhibitors of nucleotide biosynthesis than are normal cells.



Methotrexate

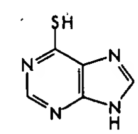
Fig. 5.

The folic acid antagonists, methotrexate and aminopterin, close structural analogs of folic acid, as antitumor agents have found clinical application in the treatment of malignant diseases, especially in the treatment of leukemia in childhood (7-10). Antifolates, folate analogs, aminopterin (4-amino folic acid) and methotrexate (amethopterin, 4-amino-10-methylfolic acid) (Fig. 5) are extremely potent competitive inhibitors of the **dihydrofolate reductase** and **thymidylate synthetase** and because of that inhibits the synthesis of RNA and DNA. Dihydrofolate reductase enzyme is needed for the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF). Dihydrofolate reductase binds methotrexate about 100 times better than dihydrofolate. Thymidylate synthetase uses methyl-tetrahydrofolic acid as a substrate and transfer methyl group to uracil present in the deoxyuridinemonophosphate (dUMP); in this transmethylation reaction deoxythymidinemonophosphate (dTMP), precursor in the biosynthetic pathway of DNA,is formed which represents the key step in the cell replication and division. Enzyme dihydrofolate reductase binds methotrexate about 100 time better than dihydrofolate. The development of drug resistance to methotrexate appears if the chemotherapy prolongs. Tumor cells that acquired MTX resistances have been found to have an increased number of DNA gene copies encoding for enzyme dihydrofolate reductase. This form of multiple gene reduplication is called gene amplification The amplified DHFR genes in MTX-resistance cells produce a markedly increased number of copies of DHFR enzyme, for exceeding the amount of MTX that can be delivered to cell, and thereby allows tumor cell DNA synthesis and tumor regrowth occurrs (11).

The application of methotrexate distrurbs the metabolism of polyamines in rapidly growing tissues. Inhibition of polyamine oxidase, the key enzyme in biodegradation pathway of spemine and spermidine, induced by methotrexate in regenerating rat liver tissue (12) is probably the consequence of the inhibition of nucleic acids and protein synthesis.

Structural analogs of purine and pyrimidine

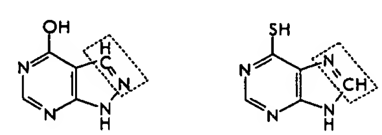
6-mercaptopurine (6-MP), the analog of hypoxanthine and adenine (Fig. 6), is a useful antitumor drug in humans. In the C_6 position of purine ring of hypoxanthine or adenine instead of NH_2 or OH group 6-MP has SH group (1,2). **6-thioguanine (6-TG)** is also thio-purine, analog of guanine. Both thioguanines, 6-MP and 6-TG are converted to nucleotide form by hypoxanthin-guanine phosphoribosyl transferase (HGPRT): their meabolites inhibit a number of enzymes in the purine pathway; some metabolites of thioguanine are incorporated into both DNA and RNA (16, 18). By incorporation and inhibition of nucleic acid synthesis this thioguanine is pariculary used in hemotherapy of malignant diseases (10, 13-16). Well known competitive inhibitor of enzyme, as a therapeutic agens in medicine is allopurinol, administrated to patients who suffer of gout. Gout is a disease of joints, usually in males, caused by an elevated concentration of uric acid in blood and tissues. The precise cause of gout is not known, but it is suspected to be due to genetic deficiency of one or another enzyme concerned in purine metabolism. The principal enzyme in this metabolism is xanthine oxidase (2).



6-Mercaptopurine

Fig. 6.

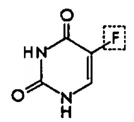
Allopurinol is structural analog of hypoxanthine (Fig. 7) and represents a competitive inhibitor of xanthine oxidase. When xanthine oxidase is inhibited the conversion of purines into uric acid is stopped; in this case the excreted products of purine metabolism are xanthine and hypoxanthine, which are more soluble in water than uric acid and less likely to form crystalline deposits (4).



Hypoxanthine (enol form) Allopurinol

Fig. 7.

5-fluororacil (5-FU) is a thymine analog in which the ring bound methyl group is substituted by fluorine (Fig. 8). The deoxynucleotide of this compound is an inhibitor of thymidilate sunthetase. 5-FU undergoes biotransformation to ribosyl and deoxyribosil nucleotide metabolites. 5-fluorouridine triphosphate is incorporated in RNA and interferes with RNA processing and function.

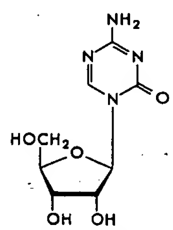


Fluorouracil

Fig. 8.

Incorporation of 5-fluorouracil into deoxyribonucleotide, 5-fluorodeoxyuridine monophosphate, results in irreversible inhibition of enzyme thymidylate sunthetase and impossibility of thymidylate (TMP) synthesis. Inhibition of thymidine monophosphate formation blocks DNA synthesis and cell multiplication (10, 16-18). 5-FU is an important anitcancer agent in the treatment of different solid tumors.

Cytosine arabinoside, ara-C, also belongs to the group of pyrimidine antagonists. This nucleoside is a specific agent of cell division S-phase especially used in acute nonlymphocyte leukemia therapy and less in the treatment of other malignant hematologic diseases. Intracellularly cytosine arabinoside is metabolised into active form, ara-CTP, which competitively inhibits DNA polymerase, thus blocking DNA synthesis (10).



5-Azacytidine

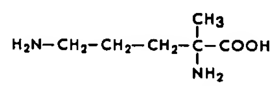
Fig. 9.

Another structure analog of cytidine is 5-azacytidine (Fig. 9). Intracellularly 5-Aza-C is metabolised into 5-aza-CTP afterwhat it is involved in DNA and RNA synthesis, damaging protein synthesis.

The possibility of application of purine and pyrimidine structural analogs as competitive inhibitors in resembling nucleotide biosynthesis is not limited only to cancer treatment. All rapidly growing cells (including bacteria and protozoa) are potentially sensitive to these agents (1).

Structural analogs of polyamines as anticancer agents

Polyamines, spermine, spermidine and putrescine are normal cell constituents. Accelerated biosynthesis and accumulation of polyamines are directly connected to cell growth and proliferation (20,21). The key enzymes in their biosynthesis are ornithine decarboxylase (ODC), which produce putrescine, and S-adenosylmethionine decarboxylase which is involved in spermidine and spermine synthesis (22). Structural analogs of ornithine α -methyl ornithine (Fig. 10) and difluoromethyl ornithine (Fig. 11) are the most used competitive inhibitors (23-25).



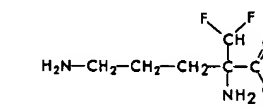
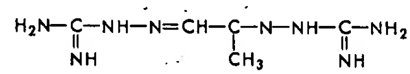


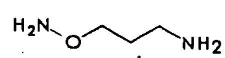
Fig 10. α-Methylornithine Fig.11. Difluoro-methylornithine

Methyl-glyoxal-bis (guanyl hidrazone), MGBG, inhibits activity of S-adenosylmethionine decarboxylase (SAMDC), the key enzyme in spermidine and spermine synthesis (26-28). The application of MGBG (Fig.12) as antiproliferative agent, is used in chemotherapy of malignant diseases; it is based on the fact that accelerated polyamine biosynthesis preceedes the accelerated nucleic acid synthesis which provides rapid cell proliferation. Blockade of polyamine synthesis slows down cellular growth and proliferation of malignant tissues (29). The new drugs in the treatment of colon cancer are highly specific and non-toxic hydroxylamine-containing competitive inhibitor of ornithine decarboxylase (ODC) 1-aminooxy-3-aminopropane (APA), structural analog of ornithine (Fig.13) and competitive inhibitor of S-adenosyl-methionine decarboxylase (SAMDC), 5'-deoxy-5'-adenosyl-methylthioethyl-hydroxylamine, (AMA) (Fig. 14), structural analog of S-adenosyl-methionine (30). This hydroxylamine - containing inhibitors od ODC and of SAMDC inhibit colon cancer cell proliferation and might be therapeutically promissing in colon cancer.



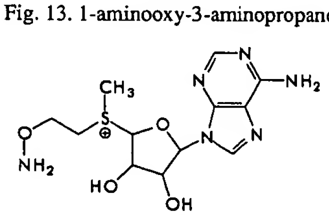
MGBG

Fig 12.



APA

Fig. 13. 1-aminooxy-3-aminopropane



AMA

Fig.14. 5'-deoxy-5'-adenosyl-methylthioethyl-hydroxylamine

The application of specific inhibitors of polyamine biosynthesis or degradation enables more detailed understanding of polyamine physiological role.

References

1. Lehninger A, Nelson D, Cox M. Principles of Biochemistry. Worth Publishers, New York, 1993.
2. Lyndal York J. Enzymes. Classification, kinetiks and control. In: Devlin MT. Wiley-Liss. Textbook of biochemistry with clinical corelations. New York, 1997: 128-174.
3. Wolinsky E. Diseases due to mycobacteria. In Cecil textbook of medicine. Wyngaarden/Smith/Bennett. 19th Edition, Saunders 1992: 1733-1745.
4. Tsai GE, Falk WE, Gunther J, Coyle TT. Improved cognition in Alzheimer,s disease with short-term D-cycloserine treatment. Am J Phychiatry 1999; 156(3): 467-469.
5. Kowabe K, Yoshihara T, Ichitani Y, Iwasaki T. Intrahippocampal D-cycloserine improves MK-801-induced memory deficits: radial-arm mase performance in rats. Brain Res 1998; 814(1-2): 226-230.
6. Saigoh K, Matsui K, Takahashi K, Nishikawa T, Wada K. The stereo-specific effect of D-serine ethylester and the D-cycloserine in ataxic mutant mice. Brain Res 1998; 808(1): 42-47.
7. Lee MW. Drug-induced hepatotoxicity. N Engl J Med 1995; 333: 1118-1127.
8. Chanarin I., Deacon R., Lumb M., Muir M., Perry J. Cobalamin-folat interrelations. A Critical Reviev. Blood 1985; 66 (3): 479-489.
9. Canal P, Chatelut E, Guichard S. Practical treatment guide for dose individualisation in cancer chemotherapy. Drugs 1998; 56(6): 1019-1038.
10. Salmon SE.S. Principles of cancer therapy. U knjizi : Wyngaarden/Smith/Bennett. Cecil textbook of medicine. 19th Edition, Saunders, 1992: 1049-1067.
11. Frei E, Jaffe N, Tattersall HNM, Pitman s, Parker L. New aproches to cancer chemotherapy with methotrexate. N Engl J Med 1975; 17: 846-851.
12. Bjelaković G, Pavlović D, Marčetić-Voinović J, Bjelaković G, Kocić G, Nikolić J, Stojanović I, Bjelaković B. Effect of methotrexate on polyamine oxidase activity in regenerating rat liver tissue. Facta Universitatis, Series "Medicine and Biology" 1995; 2(1): 10-13.
13. Bokkerink PMJ, DeAbreu AR, Bakker AHM, Hulscher WT, van Baal MJ, de Vaan AMG. Dose related effects of methotrexate on purine and pyrimidine nucleotides and on cell-kinetic parameters in molt-4 malignant human T-lymphocblasts. Biochem Pharmacol 1986; 35: 3557-3564.
14. Innocenti F, Danesi R, Bocci G, Foglis S, Di Paolo A, Del-Tacca M. Metabolism of 6-mercaptopurine in erythrocytes, liver and kidney of rats during multiple-dose regiments. Cancer Chemother Pharmacol 1999; 43(2): 133-140.
15. Lenard L. Clinical implications of thiopurine methyltransferase-optimization of drug dosage and potential grug interactions. Ther Drug Monit 1998; 20(5): 527-531.
16. Balis FM, Holcenberg JS, Poplack DG, Ge J, Sother HN, Murphy RF, Ames MM, Waskerwitz MJ, Tubergen DG, Zimm S, Gilchrist GS, Bleyer WA. Pharmacocinetics and pharmacodynamics of oral methotrexate and mercaptopurine in children with lower risk acute lymphoblastic leukemia: a joint children,s cancer group and pediatric oncology branch study. Blood 1998; 92(10): 3569-3577.
17. Thomas DM, Zalcberg JR.5-fluorouracil: a pharmacological paradign in the use of citotoxics. Clin Exp Pharmacol Physiol 1998; 25(11): 887-895.
18. Mader RM, Muller M, Steger GG. Resistance to 5-fluorouracil. Gen Pharmacol 1998; 31(5): 661-666.
19. Codacci-Pisanelli G, Krazovansky J, van-der Wilt CL, Noodhuis P, Colofiore JR, Martin DS, Franchi F, Peters GJ. Modulation of 5-fluorouracil in mice using uridine diphosphoglukose. Clin Cancer Res 1997; 3(2): 309-315.
20. Tabor WA, Tabor H. Polyamines. Ann Rev Biochem 1984; 53: 749-790.
21. Snyder HS, Russell HD. Polyamine synthesis in rapidly growing tissues Fed Proc 1970; 29: 1575-1582.
22. Morgan MLD. Polyamines. Assays Biochem 1987; 23: 82-115.
23. Filliname HR, Morris RD. Accumulation of polyamines and its inhibition by methyl glyoxal bis-(guanylhydrasone) during lymphocytes transformation. Polyamines in Normal and Neoplastic Growth.Edited by DH Russell. Raven Press, New York 1973: 249-260.
24. Harik IS, Snyder HS. Ornithine decarboxylase:inhibition by α-hydrasinoornithine. Biochim Biophys Acta 1973; 327: 501-509.
25. Holtta E, JanneJ, Hovi T. Suppression of the formation of polyamines and macromolecules by DL-α-difluoromethylornithine and methylglyoxal bis (guanylhydrasone) in phytohaemagglutinine-activated human lymphocutes. Biochem J 1979; 178: 109-117.
26. Williams-Ashman GH, Seidenfeld J. Aspects of the biochemical pharmacology of methylglyoxal bis (guanylhydrasone). Biochem Pharmacol 1986; 35(6): 1217-1225.
27. Janne J, Morris R. Inhibition of S-adenosylmethionine decarboxylase and diamine oxidase activities by analoges of methylglyoxal bis(guanylhydrasone) and their celluar uptake during lymphocyte activation. Biochem J 1984; 21(8): 947-951
28. Seppanen P, Alhonen-hongisto L, Janne J. Relation of the antiproliferative action of methylglyoxal-bis(guanylhydrasone) to the natural polyamines. Eur J Biochem 1980; 110: 7-12.
29. Bennet J, Ehrki J, Fadale P, Dave C, Mihich E. Immunosupresive effects of methylglyoxal-bis (guanylhydrasone) on mouse bone marrow and spleen cells and their antagonism by spermidine. Biochem Pharmacol 1978; 27: 1555-1560.
30. Milovic V, Turchanowa L, Khomutov RA, Khomutov MR, Caspary FW, Stein J. Hydroxylamine-containing inhibitors of polyamine biosynthesis and impairment of colon cancer cell growth. Biochem Pharmacol 2001; 61: 199-201.

KOMPETITIVNI INHIBITORI ENZIMA I NJIHOVA TERAPIJSKA PRIMENA

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Kratak sadržaj: *Enzimi katalizuju gotovo sve biohemijske procese u ćeliji. Primena mnogih lekova u medicini, antimetabolita, bazirana je na konceptu kompetitivne enzimske inhibicije. Antimetaboliti se veoma malo strukturalno razlikuju od prirodnih enzimskih supstrata. Svoja specifična dejstva ispoljavaju ponašajući se kao kompetitivni inhibitori određenih enzimskih reakcija blokirajući formiranje neželjenih metabolita u organizmu.U antimetabolite spadaju antibakteriski, antivirusni i antitumorski lekovi. Najzastupljeniji od njih jesu sulfonamidi, strukturni analozi amino kiselina (cikloserin, 5-fluoroalanin), antagonisti folne kiseline (4-amino-10-metil folna kiselina -metotreksat), analozi purina i pirimidina (6-merkaptopurin, alopurinol, 5-fluorouracil; 5-azacitidin), inhibitori biosinteze poliamina (α-difluorometil ornitin, metillglioksal-bis (guanil hidrazone=MGBG).*
Primena enzimskih inhibitora, antimetabolita,osim terapiskog značaja, omogućava bolje razumevanje raznih metaboličkih puteva, kao i bolje upoznavanje mehanizma delovanja enzima.

Ključne reči: *Enzimska inhibicija, sulfonamidi, antimetaboliti, antifolati, analozi purina i pirimidina, analozi poliamina, hemioterapija*

Stroke

(*Stroke.* 1997;28:580-583.)

Articles

Perindopril Reduces Blood Pressure but Not Cerebral Blood Flow in Patients With Recent Cerebral Ischemic Stroke

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Abstract

Background and Purpose The relationship between high blood pressure and the incidence of stroke is well established. Currently the effects of lowering blood pressure in patients with established cerebrovascular disease is undetermined, and there is continuing concern regarding the treatment of patients soon after a stroke event. Angiotensin-converting enzyme inhibitors maintain cerebral blood flow despite lowering blood pressure in patients with heart failure and otherwise uncomplicated hypertension. We tested the hypothesis that perindopril, an angiotensin-converting enzyme inhibitor with a gradual onset of action and a minimal first-dose hypotensive effect, lowers blood pressure without adversely affecting cerebral blood flow in patients 2 to 7 days after symptoms of cerebral infarction.

Methods Patients were randomized to receive 15 days of oral perindopril (4 mg) or placebo in a double-blind study. Blood pressure was monitored semiautomatically. Cerebral blood flow was calculated from internal carotid artery and vertebral Doppler ultrasound, supplemented by middle cerebral artery blood velocities.

Results Twenty-four patients completed the protocol; four additional patients were withdrawn for reasons unrelated to treatment. Patients on perindopril had a placebo-corrected reduction in blood pressure of 19/11 mm Hg. Blood pressure remained reduced after 2 weeks of treatment. In contrast, total cerebral blood flow was unaffected by perindopril. Neurological symptoms improved similarly in both groups.

Conclusions Perindopril was well tolerated and effectively reduced blood pressure without reducing carotid territory blood flow in patients with symptoms of recent cerebral ischemia.

Key Words: angiotensin-converting enzyme inhibitors • cerebral blood flow • Doppler • hypertension

Introduction

Blood pressure is an established risk factor for the primary incidence of stroke. A reduction of 5 mm Hg confers a population risk reduction of stroke incidence of 30%.[1] The potential benefit of antihypertensive therapy after cerebral infarction is undefined, but it is likely that treatment will be of most benefit in those patients with a higher risk of future stroke, ie, those with underlying cerebrovascular disease. A definitive trial recruiting sufficient numbers of patients to demonstrate the efficacy of antihypertensive therapy as secondary prevention has not yet been performed, but a large, randomized, multicenter, placebo-controlled study using perindopril and/or a thiazide diuretic (PROGRESS) will enroll from 6000 to 8000 patients with cerebrovascular disease and mild or moderate hypertension. It is hoped that this study will clarify the relationship between BP and the secondary incidence of stroke. Perindopril is an ACE inhibitor with a gradual onset of action and a relatively long half-life allowing once daily dosing; it is less likely to cause first-dose hypotension than other shorter-acting preparations such as captopril or enalapril.[2] ACE inhibitors may be particularly suited to patients with cerebrovascular disease because they do not adversely affect cerebral blood flow.[3]

Lowering BP within hours of acute stroke can lead to dramatic neurological deterioration, probably by reducing cerebral perfusion to the infarct zone.[4] [5] The Intravenous Nimodipine West European Stroke Trial (INWEST) evaluated the effects of the calcium channel blocker nimodipine in patients within 72 hours of acute stroke. Increased mortality was associated with a reduction in BP in actively treated patients.[6] A BP-lowering effect was also correlated with a poor clinical outcome in a phase II study of the ion channel blocker lifarizine.[7] In the first few days after acute stroke, cerebral autoregulation and local cerebral perfusion are deranged, and therefore any change in systemic BP may cause a critical reduction in local cerebral perfusion. In most cases these changes normalize within 3 to 4 days, and cerebral autoregulation is restored.[8] Immediate BPs are often elevated in patients with acute stroke and resolve within several days of hospital admission.[9] It would therefore seem prudent to defer consideration of patients for

antihypertensive therapy for at least 72 hours after hospital admission. After this time, it is still unclear which patients should receive antihypertensive therapy and exactly when this should be instituted.

Subjects and Methods

A double-blind, randomized trial design compared 15 days of oral perindopril (4 mg/d) with placebo in patients admitted to our stroke unit with a clinical and CT diagnosis of cerebral ischemia. Patients with normal CT scans were included in the study since CT is insensitive to early signs of infarction and to small subcortical infarcts. All patients had mild to moderate hypertension (170 to 250/95 to 120 mm Hg) as defined by two BP readings within the inclusion range at least 6 hours apart within the 24 hours before entry into the study. BPs at the time of drug administration were therefore not identical to screening BP readings because the latter were recorded in the hour immediately before drug dosing. The clinical and CT stroke classifications, incidence of previously diagnosed or treated hypertension or cerebrovascular disease, and carotid stenosis on Doppler ultrasound are documented in Table 1. Demographics are summarized in Table 2.

View this table: **Table 1.** Clinical Details of Patients at Entry to Study
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View this table: **Table 2.** Demographic Details of Patients at Entry to Study
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Patients with severe carotid disease were excluded from the study for technical and safety reasons. Patients admitted on prescribed antihypertensive therapy had treatment discontinued according to local treatment guidelines for at least 48 hours before entry into the study. Ethical approval was obtained from the West ethical committee, and patients gave written informed consent to participate. Clinical and neurological assessments according to the NIH Stroke Scale[10] were made before study entry and repeated on day 15. BP was measured semiautomatically with the use of Marquette oscillometric equipment (Marquette Electronics) before treatment and then hourly up to 10 hours after first dosing. BP measurement was repeated at 24 hours and at 2 weeks. Total cerebral blood flow was calculated from bilateral internal carotid artery Doppler ultrasound (Acuson 128, 5-MHz probe) coupled to a wall tracker device (Wall Track System, Neurodata). Arterial flow was calculated as (πxdiameter^{2}xmean velocity)/4. Details of Doppler methods used have been published previously.[11] MCA velocity and resistance index were

measured by transcranial Doppler (Nicolet EME TC2000, 2-MHz probe). Doppler recordings were undertaken before treatment and at 2, 4, 8, and 24 hours and repeated at 2 weeks. An additional recording of MCA velocity was made at 6 hours. Routine safety biochemistry and hematology data were collected at entry and at the conclusion of the study period. Plasma renin activity, angiotensin II activity, ACE activity, and drug plasma levels were assessed at 0, 4, 6, 8, 12, and 24 hours and at 2 weeks.

Laboratory Measurements
Plasma renin activity was measured by radioimmunoassay of generated angiotensin I (detection limit, 0.54 ng/mL per hour; coefficient of variation, 6.7%). Angiotensin II was determined according to Morton and Webb[12] (detection limit, 2.0 pg/mL; coefficient of variation, 6.4%). ACE was assayed by incubation of plasma/serum with the ACE substrate analogue hippuryl-histidyl-leucine. The hippuric acid produced was extracted and then quantified with the use of high-performance liquid chromatography. When this assay is used, the limit of quantification is 0.05 mmol/L, and the limit of detection is 0.01 mmol/L.

Perindopril levels were assessed by the direct determination of ACE inhibitor in plasma by radioenzymatic assay with a modification of the method of Reydel-Bax et al[13] and liquid chromatography-assisted assay for ACE in serum. The active metabolite perindoprilat is measured with a calibration range of 0.16 to 20 ng/mL. The limit of quantification is 0.16 ng/mL, and the limit of detection is 0.1 ng/mL.

Statistical Analysis
Results were analyzed by repeated measures ANOVA and ANCOVA with the use of Statistica for Windows software (Statsoft, version 51994). With a sample size of 24 patients, we expected to detect a difference in cerebral blood flow of 16% with 80% power.

Results

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Tolerance and Safety
A total of 28 patients were recruited to the study with 24 completing the protocol. Patients were aged between 52 and 89 years. Four patients failed to complete the protocol. One patient was withdrawn after an adverse event that was not believed to be related to drug action. This event consisted of transient left arm paresthesia while the patient was undergoing carotid Doppler imaging of the right internal carotid artery 9 hours after perindopril dosing; symptoms lasted 5 minutes and did not recur. Another patient in the perindopril group was withdrawn after only one dose when his renal function was found to be mildly impaired before drug treatment. Two patients receiving placebo did not complete the study: one was lost to follow-up after transfer to an outlying hospital, and another had inadequately documented data to allow analysis. All withdrawn patients were followed up and were well at the conclusion of the study.

Perindopril was therefore well tolerated with no serious adverse events. Biochemistry and hematology results were unremarkable. Mean NIH scores in placebo and treatment groups improved in a clinically and statistically similar manner but with no difference between the two groups (Table 2).

Systolic, diastolic, and mean BPs were significantly reduced in the perindopril-treated patients from 2 to 24 hours after perindopril (*P*<.004) and remained reduced after 2 weeks of treatment (perindopril group: 168±17/91±9 mm Hg at baseline to 150±21/79±14 mm Hg at 4 hours; placebo group: 172±26/92±14 mm Hg at baseline to 173±23/91±13 mm Hg at 4 hours; ie, a placebo-corrected reduction of 18/11 mm Hg). There was no associated change in heart rate in either group. Despite the reduction in BP, there was no reduction in total internal carotid artery flow or MCA velocity, even at the time of peak drug effect (Figure). Internal carotid artery flow was increased at 8 hours in the perindopril-treated patients (*P*<.004). Neither common nor external carotid artery flow was significantly different between treatment and placebo groups. Determinations of velocity and blood vessel diameter in common, internal, and external carotid vessels similarly showed no difference between perindopril and placebo groups. In addition, there was no difference in the MCA resistance index (a measure of artery tone and distensibility). Renin activity and angiotensin II levels were not significantly different between perindopril and placebo groups, but ACE was inhibited by perindopril (*P*<.001). The AUC $_{0\text{-}24}$ for perindoprilat was 135 h·ng/mL (data not shown).

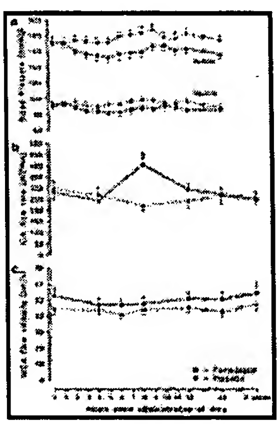

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Figure 1. a, Systolic and diastolic BP vs time. BP was significantly reduced by perindopril from 2 to 24 hours after perindopril (*P*<.004) and remained reduced after 2 weeks of treatment. b, Internal carotid artery (ICA) flow rate vs time. There was an increase in the perindopril-treated patients at 8 hours (*P*<.004) but no significant changes at other time points. c, MCA flow velocity vs time. No significant deviation from baseline was detected. All error bars represent SE of the mean.

Discussion

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Perindopril was well tolerated in patients when administered after an acute ischemic stroke. The study was not designed to demonstrate any

long-term effect on neurological outcome, but the results are reassuring since no patient suffered a drug-associated neurological deterioration.

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ACE inhibitors are thought to lower BP without adversely affecting total cerebral blood flow. The role of angiotensin in the physiological control of the cerebral circulation has not been adequately defined. The configuration of the ACE gene may be important in the generation of accelerated atherosclerosis in the coronary and cerebral circulations, although there is conflicting evidence that ACE genotype is relevant in the development of cerebrovascular disease. Angiotensin II receptors regulate cerebral blood flow in rats. Large cerebral arteries containing angiotensin II receptors ameliorate increases in blood flow in response to a rise in BP.[14] Treatment of hypertensive animals with ACE inhibitors resets cerebral autoregulation at a lower level, but this effect may be shared with other antihypertensive agents. In hypertensive humans without a history of stroke, captopril increases cerebral blood flow, measured by a SPECT scanning radionuclide ^{133}Xe technique, with an inverse correlation between reduction in BP and mean cerebral blood flow.[15]

Two single-dose studies in healthy volunteers[16] [17] assessing blood flow with carotid and transcranial Doppler after ACE inhibitor administration demonstrated results similar to those in our study, with BP effectively lowered and bilateral common carotid artery flow increased. MCA flow velocity was unchanged, but there was an increase in cerebral vascular resistance index, suggesting vasoconstriction in the cerebral arterioles.[16]

Hypertensive stroke patients have only been assessed in two uncontrolled studies (each recruiting 12 patients). Both studies used SPECT scanning and a ^{133}Xe inhalation technique. In one study the drug effectively lowered BP and increased cerebral blood flow to both hemispheres,[17] while in the other study a fall in BP was not associated with a significant blood flow effect.[18]

Doppler data support the hypothesis that perindopril does not adversely affect cerebral blood flow or alter cerebral hemodynamics in a clinically significant way. The results, however, cannot be considered relevant to all patients with severe carotid disease. It is conceivable that the presence of hemodynamically significant carotid lesions may lead to a reduction in cerebral perfusion distal to a site of stenosis after the lowering of systemic BP. This may be particularly relevant in the hours and days immediately after acute stroke, when cerebral autoregulation is deranged and consequently perfusion is directly dependent on systemic BP levels. We did not consider it ethical to treat patients before 48 hours of onset of stroke symptoms since there is good trial evidence that lowering BP at this time results in adverse outcome.[6] [7] Further research is required to assess whether these patients are indeed more prone to neurological deterioration after BP reduction before treatment guidelines can be advised. It is also possible that while total internal carotid artery flow is preserved, local ischemic areas may become increasingly compromised as a result of a reduction in BP. Other forms of brain imaging techniques such as SPECT or positron emission tomography scanning may provide further information on the effects of BP-lowering treatment on regional perfusion, particularly in the area surrounding the cerebral infarct.

Our data suggest that starting perindopril treatment within 2 and 7 days of the onset of cerebral ischemia can successfully and safely lower BP without adversely affecting total cerebral blood flow in patients without severe carotid stenosis.

Selected Abbreviations and Acronyms

ACE	= angiotensin-converting enzyme
BP	= blood pressure
MCA	= middle cerebral artery
NIH	= National Institutes of Health
SPECT	= single-photon emission computed tomography

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References

1. Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, Godwin J, Qizilbash N, Taylor JD, Hennekens CH. Blood pressure, stroke, and coronary heart disease, part 2: short-term reductions in blood pressure: overview of randomized drug trials in their epidemiological context. *Lancet.* 1990;335:827-839. [Medline]
2. MacFadyen RJ, Lees KR, Reid JL. Differences in first dose response to angiotensin converting enzyme inhibition in congestive heart failure: a placebo controlled study. *Br Heart J.*. 1991;66:206-211. [Abstract]
3. Waldemar G, Vorstrup S, Andersen AR, Petersen H, Paulson OB. Angiotensin converting enzyme inhibition and regional cerebral blood flow in acute stroke. *J Cardiovasc Pharmacol.* 1989;14:722-729. [Medline]
4. Yatsu FM, Zivin J. Hypertension in acute ischemic strokes; not to treat. *Arch Neurol.*. 1985;42:999-1000. [Medline]
5. Strandgaard S. Cerebral ischaemia caused by over zealous blood pressure lowering. *Dan Med Bull.* 1987;34(suppl 1):5-7.
6. Wahlgren NG, MacMahon OG, DeKeyser J, Indredavik B, Ryman T, for the INWEST Study Group. Intravenous Nimodipine West European Stroke Trial (INWEST) of nimodipine in the treatment of acute ischaemic stroke. *Cerebrovasc Dis.*. 1994;4:204-210.
7. Squire IB, Lees KR, Pryse-Phillips W, Kertesz A, Bamford J. Lifarizine study group: a

pilot safety study. *Cerebrovasc Dis.*. 1996;6:156-160.
8. Fiesc hi C, Lenzi GL. Cerebral blood flow and metabolism in stroke patients. In: Russell RW, ed. *Vascular Diseases of the Central Nervous System.* 2nd ed. New York, NY: Churchill Livingstone, Inc; 1983:101-127.
9. C arlberg B, Asplund K, Haag E. Course of blood pressure in different subsets of patients with acute ischaemic stroke. *Cerebrovasc Dis.*. 1991;1:281-287.
10. Brott T, Adams HP, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Herzberg V, Rorick M, Moomaw CJ, Walker M. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke.* 1989;20:864-870.[Abstract]
11. Grosset DG, Muir KW, Lees KR. Systemic and cerebral hemodynamic responses to the non-competitive NMDA antagonist CNS 1102. *J Cardiovasc Pharmacol.* 1995;25:705-709. [Medline]
12. Morton JJ, Webb DJ. Measurement of plasma angiotensin II. *Clin Sci.*. 1985;68:483-484. [Medline]
13. Reydel-Bax P, Redalieu E, Rakhit A. Direct determination of angiotensin converting enzyme inhibitors in plasma by radioenzymatic assay. *Clin Chem.*. 1987;33:549-553. [Abstract]
14. Stromberg C, Naveri L, Saavedra JM. Angiotensin AT2 receptors regulate cerebral blood flow in rats. *Neuroreport.* 1992;3:8703-8704.
15. Minematsu K, Yamaguchi T, Tsuchiya M, Ito K, Ikeda M, Omae T. Effect of the angiotensin converting enzyme inhibitor captopril on cerebral blood flow in hypertensive patients without a history of stroke. *Clin Exp Hypertens.*. 1987;9:551-557.
16. Demolis P, Carville C, Giudicelli J-F. Effects of an angiotensin converting enzyme inhibitor, lisinopril, on cerebral blood flow autoregulation in healthy volunteers. *J Cardiovasc Pharmacol.* 1993;22:373-380. [Medline]
17. Naritomi H, Shimizu T, Watanabe Y, Murata S, Sawada T. Effects of the angiotensin converting enzyme inhibitor alacepril on cerebral blood flow in hypertensive stroke patients: a pilot study. *Curr Ther Res Clin Exp.*. 1994;55:1446-1454.
18. Waldemar G, Schmidt JF, Anderson AR, Vorstrop S, Ibsen H, Paulson OB. Angiotensin converting enzyme inhibition and regional cerebral blood flow in acute stroke. *J Cardiovasc Pharmacol.* 1989;14:722-729.[Medline]

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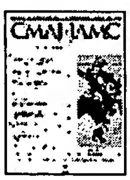

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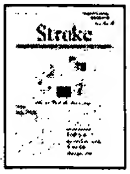

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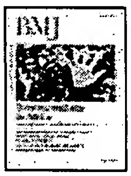

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